

# Serum chitotriosidase enzyme activity in patients with Crimean-Congo hemorrhagic fever

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## Abstract

**Background:** Crimean-Congo hemorrhagic fever (CCHF) is a public health problem in many countries. Chitotriosidase (ChT) is an enzyme secreted by activated macrophages that catalyzes the hydrolysis of chitin and chitin-like substrates. The goal of this study was to assess the relationship between serum ChT activity and mortality.

**Methods:** ChT activities on the first day of hospitalization were analyzed in serum from 46 patients with CCHF and 36 healthy controls. Serum ChT activities and other clinical and laboratory parameters for patients with non-fatal and fatal CCHF were compared.

**Results:** The median ChT activity was increased in all patients with CCHF [189.9 (134.8–246.6) nmol/mL/h]. The median ChT activity in the non-fatal CCHF group [220.2 (180.6–290.1) nmol/mL/h] was higher compared with the fatal CCHF group [29.2 (16.5–45.7) nmol/mL/h] ( $p < 0.001$ ). In univariate analysis, platelet count, lactate dehydrogenase (LDH), and activated partial thromboplastin time were associated with mortality.

**Conclusions:** This is the first study investigating the association of serum ChT enzyme activity with mortality from CCHF. This study suggested that relatively low ChT enzyme activities may be a prognostic marker in patients with CCHF.

Clin Chem Lab Med 2009;47:1543–7.

**Keywords:** chitotriosidase enzyme activity; Crimean-Congo hemorrhagic fever; mortality.

## Introduction

Crimean-Congo hemorrhagic fever (CCHF) was first described clinically in 1944 in Crimea of the former Soviet Union during a large outbreak of over 200 cases. The CCHF virus, belonging to the genus *Nairovirus* of *Bunyaviridae* family, was identified in 1967 from a patient in Uzbekistan, and was found to be similar to a virus isolated in 1956 in Congo, and therefore, named Crimean-Congo (1). The virus is transmitted to humans through the bite of Ixodid ticks or by contact with blood or tissues from infected livestock (2). CCHF is not highly contagious. However, there is a risk for spread from person to person by exposure to infected blood, respiratory secretions and excreta, resulting occasionally in nosocomial outbreaks (3).

CCHF is potentially fatal and found in more than 30 countries in Africa, Asia, southeast Europe, and the Middle East (4, 5). The virus infects the reticuloendothelial system leading to a sudden onset of symptoms. Shock and increased vascular permeability are probably a consequence of hyperactivation of the cytokine pathways (6). Hemorrhagic complications may also be related to hepatic damage and coagulopathy (7).

In humans, infection with tickborne CCHF virus often results in a serious illness followed by death. It has been estimated that one-third of patients hospitalized with CCHF die, although many recover rapidly after a febrile illness (4). After a brief incubation period, the patient has sudden onset of fever, myalgia, nausea and severe headache. Within 3–6 days of the onset of illness, development of a petechial rash and hemorrhagic symptoms, such as epistaxis, hematemesis, and melena may occur. The most extremely ill patients develop multiorgan failure characterized by shock, hemorrhage and coma (8). Mortality rates range from 15% to 60% (5).

The pathogenesis of CCHF is not well understood. The vascular endothelium is both a direct and indirect target of all hemorrhagic viruses. Mononuclear phagocytes are also targets for hemorrhagic viruses. They are activated upon infection and release certain cytokines and chemokines. These mediators indirectly target the endothelium. Serum concentrations of proinflammatory cytokines including interleukin (IL)-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were higher in patients with fatal CCHF (9).

Chitin is an unbranched polymer formed by  $\beta$ -1,4-linked N-acetyl-D-glucosamine units found in insects, fungi and egg shells of some nematodes; it is not thought to occur in mammals. Catabolism of chitin is mediated by chitinases (O-glycosyl hydrolases (EC 3.2.1.-)). Mammalian chitinases are all related to Family 18 chitinases, with Family 19 chitinases found

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Received March 25, 2009; accepted September 8, 2009

primarily in plants. Chitotriosidase (ChT), also known as macrophage chitinase, is not expressed in monocytes, but is synthesized by activated macrophages in a number of pathological settings (10, 11). It has ability to cleave chitotriose, the trimeric form of  $\beta$ -1,4-N-acetyl-D-glucosamine (12), and is able to catalyze the hydrolysis of chitin-like substrates, such as 4-methylumbelliferyl chitotrioside (13). It was recently reported that leukocytes may also secrete plasma ChT under physiological conditions (14). It is secreted predominantly into blood, and ChT activity is an established biochemical marker of macrophage accumulation in several lysosomal diseases, especially Gaucher's disease (10, 11). The function of ChT in humans is unknown. Approximately 6% of European Caucasians are deficient in ChT activity due to a common 24-base pair duplication in exon 10 of the *ChT* gene. No clinical abnormality has been associated with this genetic defect (15).

The goal of this study was to assess the relationship between serum ChT activity with mortality due to CCHF.

## Materials and methods

### Study design and subjects

We studied 38 patients with non-fatal CCHF (14 females and 24 males), and eight patients (4 females and 4 males) with fatal CCHF admitted to The Ankara Numune Education and Research Hospital (ANERH) during the spring and summer of 2006. All patients with positive IgM and/or PCR results for CCHF virus in blood participated in the study.

For all patients, only CCHF virus was found as the causative agent. Analyses for other infectious agents were negative, including *Leptospira*, *Salmonella*, *Rickettsia*, *Brucella*, and *Toxoplasma* species, *Coxiella burnetii*, agents of Lyme disease and malaria, rubella and herpes viruses, cytomegalovirus, hepatitis A, B and C viruses. Thirty-six healthy controls (15 females and 21 males) with no clinical evidence of CCHF were also included. All patients were given supportive treatment only, including erythrocytes, fresh frozen plasma and whole blood preparations, depending on the state of their hemostasis. Following admission to the hospital, antibiotics, such as doxycycline and ciprofloxacin were given for empirical treatment of common regional zoonotic diseases. The study protocol was approved by the Ethical Committee of the Ankara Numune Education and Research Hospital, Ankara, Turkey. Written informed consent was obtained from patients or their family members and control subjects.

### Samples

Serum was collected on the first day and last day (day of death for fatal CCHF or discharge day for non-fatal CCHF) of hospitalization for each patient. All sera were stored at  $-80^{\circ}\text{C}$  until analysis.

Age, gender, duration of symptoms, history of tick bites, presence of fever, myalgia, bleeding, white blood cell (WBC) count, platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT) and fibrinogen, measured on the first day of hospitalization, were recorded. Serum ChT activity on the first day

of hospitalization in patients with CCHF was compared with serum ChT activity in healthy controls. We also compared serum ChT activities and other clinical and laboratory parameters in the two groups of patients with non-fatal and fatal CCHF.

### ChT enzyme assay

Analyses for serum ChT enzyme activity were performed according to the method described by Tunc et al. (16). Briefly, 25  $\mu\text{L}$  of serum was incubated with 100  $\mu\text{L}$  of 22  $\mu\text{mol/L}$  4-methylumbelliferyl- $\beta$ -D-N,N,N-triacetylchitotriose in McIlvaine's phosphate-citrate buffer, pH: 5.2, for 1 h at  $37^{\circ}\text{C}$ . The reaction was terminated by the addition of 120  $\mu\text{L}$  0.5 mol/L  $\text{Na}_2\text{CO}_3$ - $\text{NaHCO}_3$  buffer, pH: 10.7, and the fluorescence of 4-methylumbelliferone was measured using a Microfluor 2<sup>®</sup> fluorimeter (Bio-Tek Instruments, Neufahrn, Germany; excitation: 355 nm, emission: 460 nm). Serum ChT activities were expressed as nanomoles of substrate hydrolyzed per milliliter per hour (nmol/mL/h). Serum ChT activities were measured in duplicate. The coefficient of variation was  $<5\%$ .

### Statistical analysis

Results were presented as medians [interquartiles (25%–75%)], frequency, and percent. Groups were compared using the  $\chi^2$ -test and Mann-Whitney U-test. Univariate and multivariate logistic regression analyses were used to determine variables associated with mortality. Statistical analysis was performed using SPSS<sup>®</sup> 11.5 Statistical Package Program for Windows (SPSS Inc, Chicago, IL, USA). Differences were considered significant at  $p < 0.05$ .

## Results

We identified one patient with ChT deficiency in patients with fatal CCHF. Clinical and laboratory features for 46 CCHF patients are summarized in Table 1. Eight of the subjects died as a result of CCHF (fatal CCHF group). Thirty-eight cases survived (non-fatal CCHF group). There were significant differences between non-fatal and fatal CCHF groups with respect to bleeding, serum ChT activity, platelet count, AST, LDH, CK, PT and aPTT. However, no significant differences were noted with respect to age, mean duration of symptoms, history of tick bites, fever, myalgia, WBC count, ALT, INR and fibrinogen. AST, LDH, CK, PT and aPTT were significantly higher in the fatal CCHF group compared with the non-fatal CCHF group. Serum ChT activity and platelet count were significantly lower in the fatal CCHF group compared with the non-fatal CCHF group.

ChT activity increased in all patients with CCHF on the first day of hospitalization ( $\geq 9.0$  nmol/mL/h), showing a median concentration of 189.9 (134.8–246.6) nmol/mL/h. The median ChT activity in healthy blood donors was 159.4 (113.4–208.2) nmol/mL/h and the median activity of ChT in the fatal CCHF group was 29.2 (16.5–45.7) nmol/mL/h. Thus, the median activity of ChT in the control group exceeded that in the fatal CCHF group by  $\sim 5$ -fold ( $p < 0.001$ ).

The median ChT activity in the non-fatal CCHF group [220.2 (180.6–290.1) nmol/mL/h] was  $\sim 7$ -fold

**Table 1** Comparison of demographic, clinical characteristics and laboratory test data between patients with non-fatal CCHF and fatal CCHF.

	Groups		p-Value
	Non-fatal CCHF	Fatal CCHF	
n	38	8	
Male, n	24 (63.2%)	4 (50%)	0.693
Age, years	42.5 (34–61)	61 (50–69)	0.074
Mean duration of symptoms, days	7 (5.0–8.25)	4.5 (3.25–9.0)	0.231
Tick-bite history, n (%)	22 (57.9%)	6 (75%)	0.453
Fever, n	34 (84.5%)	7 (87.5%)	0.870
Myalgia, n	35 (92.1%)	8 (100%)	1.000
Bleeding, n	10 (26.3%)	8 (100%)	<0.001
Chitotriosidase activity <sup>a</sup> , nmol/mL/h	220.2 (180.6–290.1)	29.2 (16.5–45.7)	<0.001
Chitotriosidase activity <sup>b</sup> , nmol/mL/h	261.8 (204.5–377.5)	33.5 (20.5–47.7)	<0.001
WBC count <sup>a</sup> , $\times 10^9/L$	2.2 (1.5–3.3)	2.3 (1.6–3.7)	0.650
Platelet count <sup>a</sup> , $\times 10^9/L$	76.5 (26.5–105.5)	15.0 (6.3–34.5)	0.003
AST <sup>a</sup> , U/L	202.5 (70–541)	780.5 (478–1060)	0.002
ALT <sup>a</sup> , U/L	90 (52–258)	220 (131–251)	0.080
LDH <sup>a</sup> , U/L	501.5 (343–789)	1587 (1158–3768)	<0.001
CK <sup>a</sup> , U/L	238 (130–803)	1148 (472–2165)	0.049
PT <sup>a</sup> , s	120 (112–131)	143.5 (123–147)	0.025
INR <sup>a</sup>	0.95 (0.87–1.04)	1.13 (0.93–1.21)	0.072
aPTT <sup>a</sup> , s	31.1 (28.4–36.9)	48.7 (41.8–82.0)	0.006
Fibrinogen <sup>a</sup> , mmol/L	2.45 (2.29–3.31)	2.50 (1.69–3.06)	0.794

Data are number (%) of patients or median [interquartiles (25%–75%)]. <sup>a</sup>On the first day of admission to the hospital. <sup>b</sup>On the last day of admission to the hospital. CCHF, Crimean-Congo hemorrhagic fever; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time.

higher compared with the fatal CCHF group [29.2 (16.5–45.7) nmol/mL/h] (Figure 1). The difference between the fatal and non-fatal CCHF groups was statistically significant ( $p < 0.001$ ). The median activities of ChT on the final day of hospitalization in the non-fatal CCHF group [261.8 (204.5–377.5) nmol/mL/h] were significantly higher than those seen in the fatal CCHF group [33.5 (20.5–47.7) nmol/mL/h] ( $p < 0.001$ ).

The mortality rate was 17.3%. Fatal cases were hospitalized a mean of 4.5 days (range 2–15) prior to death. The duration of hospitalization was ~7 days (range 2–24) in non-fatal cases.

Other parameters analyzed for mortality are shown in Table 2. In univariate analysis, platelet count, LDH and aPTT were associated with mortality (Table 2).

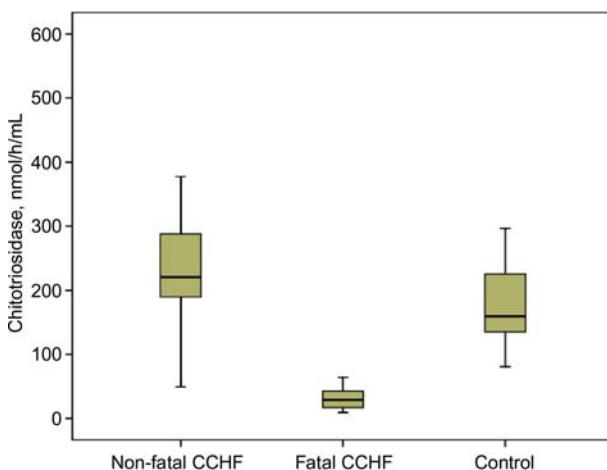
However, in multivariate analysis none of the variables was associated with mortality.

## Discussion

Chitinases are ubiquitous chitin fragmenting enzymes present in various organisms and involved in several biological processes including defense against chitin containing pathogens, such as fungi. Human ChT is a fully active chitinase (17). It is now known that activated macrophages are the main source of ChT. The ChT enzyme can be considered an inflammatory protein since is only secreted by activated macrophages. It does not behave as an acute reactive protein but rather as a marker of chronic inflammation (18).

This enzyme is involved in the degradation of chitin containing pathogens. Little is known about physiological role of ChT and the mechanisms that could modify its activity in humans, but these might be relevant in clinical practice. High serum ChT activity in patients with atherosclerosis demonstrates in vivo the presence of activated macrophages (10).

The activating factors are still unknown, and are likely to be different in the disorders studied. Increases have been observed in various lysosomal disorders, such as malaria, thalassemia and fungal infections (19–23). In addition, increased activity has been reported in tissues and plasma of guinea pigs infected by *Aspergillus fumigatus*, and in plasma and urine of a neonate with *C. albicans* infection (23, 24). As increased activities of ChT have been described in neonates with bacterial infections (17), this may suggest that the spectrum of anti-microbial action of ChT

**Figure 1** Serum chitotriosidase activities in non-fatal and fatal CCHF patients compared to control subjects.

**Table 2** Univariate analysis of risk factors for mortality of patients with CCHF.

	OR	95% CI	p-Value
Fever	0.82	0.08–8.53	0.87
Myalgia	NA	NA	NA
Bleeding	NA	NA	NA
Tick-bite history	2.18	0.39–12.35	0.38
Chitotriosidase activity <sup>a</sup> , nmol/mL/h	0.887	0.768–1.024	0.103
WBC count <sup>a</sup>	1.00	1.00–1.001	0.36
Platelet count <sup>a</sup>	0.95	0.92–0.99	0.02
AST <sup>a</sup>	1.001	1.000–1.002	0.06
ALT <sup>a</sup>	1.000	0.998–1.003	0.84
LDH <sup>a</sup>	1.003	1.001–1.005	0.01
CK <sup>a</sup>	1.000	1.000–1.001	0.18
PT <sup>a</sup>	1.038	0.997–1.081	0.07
INR <sup>a</sup>	1.035	0.999–1.081	0.13
aPTT <sup>a</sup>	1.005	1.001–1.009	0.02
Fibrinogen <sup>a</sup>	0.998	0.988–1.007	0.63

<sup>a</sup>On the first day of admission to the hospital. CCHF, Crimean-Congo hemorrhagic fever; OR, odds ratio; NA, non-applicable; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time.

extends to bacteria, thus playing a complementary role to lysozyme. Lysozyme has a well-documented action against bacteria but less is known about its anti-fungal activity (25, 26). However, data from another study do not support this view (12).

To our knowledge, there is no information concerning the association between serum ChT activity with CCHF. Our study is the first clinical study to assess this relationship in patients with CCHF, as well as being the first study to investigate the association of serum ChT activity with mortality from CCHF.

In the studies analyzing risk factors in patients with CCHF, parameters during the first 5 days of illness were studied (27). However, we used parameters measured on the first day of admission to predict mortality. Bakir et al. reported that INR, AST, LDH, and CK were higher in patients who died (28). In their study, serum ChT activities were significantly lower in patients with fatal CCHF compared with non-fatal CCHF and healthy controls. Serum ChT could be a prognostic marker in patients with CCHF. Different predictors of fatality were reported as risk factors for CCHF (27, 29–32), and those previously defined severity criteria were not always consistent. These criteria include decreased platelet counts, prolonged aPTT, increased AST, ALT and LDH activities in a univariate analysis from five different studies. As ANERH is a referral and tertiary-care hospital, more complicated cases are transferred to this facility from other hospitals. The overall mortality rate of CCHF disease was 17.3% in this study. Our mortality rate is higher than the average for Turkey (5.4%) (32). In the current study, decreased platelet counts, increased LDH and prolonged aPTT were identified as predictors of mortality in univariate analysis. However, in multivariate analysis, none of the variables was found to be statistically significant. This may be due to the limited numbers of fatal CCHF cases.

In conclusion, the present study suggested that relatively low ChT enzyme activity may be a prognostic marker in patients with CCHF. This is the first study investigating the association of serum ChT enzyme

activity with mortality in CCHF. In this study, we report that low serum ChT enzyme activity can be detected in patients with fatal CCHF. In addition, we detected high and normal serum ChT activities in patients with non-fatal CCHF. This finding suggests that ChT may have an antiviral feature or a protective effect against secondary bacterial infections during CCHF. Further studies with larger numbers of patients are required to clarify this hypothesis and to confirm these promising but preliminary results.

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